

09/622,353

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L1 and primer\$1	▲
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<u>L3</u>	L2 and ((adapter\$1 or adaptor\$1 or linker\$1 or oligo\$2)near5 hybridiz\$5)
<u>L2</u>	L1 and primer\$1
<u>L1</u>	(unknow\$3 or unidentif\$3) near10 transpos\$4

1	<u>L3</u>
11	<u>L2</u>
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L3: Entry 1 of 1

File: USPT

Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6300071 B1*might be prior art*

TITLE: Method for detecting nucleic acid methylation using AFLP.TM.

Brief Summary Text (5):

(c) contacting said tagged restriction fragments under hybridizing conditions with at least one oligonucleotide primer;

Brief Summary Text (6):

(d) amplifying said tagged restriction fragments hybridized with said primers by PCR or similar technique so as to cause further elongation of the hybridized primers along the restriction fragments of the starting DNA to which said primers hybridized; and

Brief Summary Text (8):

The amplified DNA-fragments thus obtained can then be analysed and/or visualised, for instance by means of gel-electrophoresis. This provides a genetic fingerprint showing specific bands corresponding to the restriction fragments which have been linked to the adapter, have been recognized by the primer, and thus have been amplified during the amplification step. The fingerprint thus obtained provides information on the specific restriction site pattern of the starting DNA, and thus on the genetic make-up of the organism from which said DNA has been derived.

Brief Summary Text (10):

The primers used in AFLP are such that they recognize the adapter and can serve as a starting point for the polymerase chain reaction. To this end, the primers must have a nucleotide sequence that can hybridize with (at least part of) the nucleotide sequence of the adapter adjacent to the 3' end of the restriction fragment to be amplified. The primers can also contain one or more further bases (called "selective bases") at the 3'-end of their sequence, for hybridization with any complementary base of bases at the 3'-end of the adapter ligated restriction fragment. As, of all the adapter ligated restriction fragments present in the mixture, only those fragments that contain bases complementary to the selective bases will subsequently be amplified, the use of these "selective" primers will reduce the total amount of bands in the final fingerprint, thus making the fingerprint more clear and more specific. Also, the use of different selective primers will generally provide differing fingerprints, which can also be used as a tool for the purposes of identification or analysis.

Brief Summary Text (12):

For a further description of AFLP, its advantages, its embodiments, as well as the techniques, enzymes, adapters, primers and further compounds and tools used therein, reference is made to EP-0 534 858, incorporated herein by reference. Also, in the description hereinbelow, the definitions given in paragraph 5.1 of EP-0 534 858 will be used, unless indicated otherwise.

Brief Summary Text (37):

The invention also comprises kits for use in the invention, comprising at least: a frequent cutter restriction enzyme; a methylation sensitive rare cutter restriction enzyme; (at least) a first and second adapter for use with the frequent cutter; and (at least) a first and second adapter for use with the rare cutter; as well as primers for use with these adapters; wherein these components are essentially as described herein.

Brief Summary Text (38):

The kits can further contain all known components for AFLP kits, such as restriction enzymes (in which case the adapters are preferably suited to be ligated to the